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Effects of two wood-based biochars on the fate of added fertilizer nitrogen—a ^{15}N tracing study

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Abstract

A ^{15}N tracing pot experiment was conducted using two types of wood-based biochars: a regular biochar and a Kon-Tiki-produced nutrient-enriched biochar, at two application rates (1% and 5% (w/w)), in addition to a fertilizer only and a control treatment. Ryegrass was sown in pots, all of which except controls received ^{15}N -labelled fertilizer as either $^{15}\text{NH}_4\text{NO}_3$ or $\text{NH}_4^{15}\text{NO}_3$. We quantified the effect of biochar application on soil N_2O emissions, as well as the fate of fertilizer-derived ammonium (NH_4^+) and nitrate (NO_3^-) in terms of their leaching from the soil, uptake into plant biomass, and recovery in the soil. We found that application of biochars reduced soil mineral N leaching and N_2O emissions. Similarly, the higher biochar application rate of 5% significantly increased aboveground ryegrass biomass yield. However, no differences in N_2O emissions and ryegrass biomass yields were observed between regular and nutrient-enriched biochar treatments, although mineral N leaching tended to be lower in the nutrient-enriched biochar treatment than in the regular biochar treatment. The ^{15}N analysis revealed that biochar application increased the plant uptake of added nitrate, but reduced the plant uptake of added ammonium compared to the fertilizer only treatment. Thus, the uptake of total N derived from added NH_4NO_3 fertilizer was not affected by the biochar addition, and cannot explain the increase in plant biomass in biochar treatments. Instead, the increased plant biomass at the higher biochar application rate was attributed to the enhanced uptake of N derived from soil. This suggests that the interactions between biochar and native soil organic N may be important determinants of the availability of soil N to plant growth.

Keywords Ammonium · Flame curtain pyrolysis · Kon-Tiki kiln · N leaching · Nitrate · Nitrous oxide

Introduction

To meet the growing food and energy demand, the application of synthetic nitrogen (N) fertilizers to agricultural soils has dramatically increased over the last century. Unfortunately, this has also caused harmful environmental effects. Specifically, the applied N is not only taken up by plants and retained in soil but is also leached, and hence causes eutrophication, when it reaches water bodies (Isermann 1990). Globally, about $55 \text{ Tg N year}^{-1}$ is leached from agricultural soils (Van Dreht et al. 2003). In addition, part of the N added to soil is emitted into the atmosphere as N_2O , causing a large impact on climate. The greenhouse gas (GHG) N_2O has a global warming potential (GWP_{100}) 265 times higher than that of carbon dioxide (CO_2) and is regarded as the single most important gas responsible for stratospheric ozone depletion (IPCC 2014; Portmann et al. 2012). The agriculture sector is responsible for 66% of gross anthropogenic N_2O emission; it has been projected that by 2050, anthropogenic N_2O

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emission will be twice as much as today (Davidson and Kanter 2014). Therefore, there is a great need for agricultural practices that can increase efficiency of applied N fertilizer use by crops while minimizing negative environmental effects such as N leaching and N₂O emissions.

The application of stable C in the form of biochars into agricultural soil has gained popularity because of its potential to sequester atmospheric C in soil. Moreover, the application of biochars to agricultural soil has potential agricultural (Biederman and Harpole 2013; Jeffery et al. 2017a) and environmental benefits such as reduced mineral N leaching and N₂O emissions (Borchard et al. 2019; Cayuela et al. 2013; Clough et al. 2013; Nguyen et al. 2017). The beneficial effects of biochars are attributed to inherent properties, which commonly include high aromaticity, porosity, specific surface area, negative surface charge, and surface charge density (Downie et al. 2009; Liang et al. 2006). These properties make biochars highly stable in soil (Kuzakov et al. 2014) and able to retain water and nutrients in soil (Glaser et al. 2002; Karhu et al. 2011; Tammeorg et al. 2014).

A fresh biochar addition may reduce the availability of nutrients to plants, particularly N, which can even reduce the crop yield (Kammann et al. 2015). However, treatment of biochars with nutrient-rich organic substances, for example through co-composting, can increase the retention and supply of plant-available nutrients, increasing the crop yield (Hagemann et al. 2017; Ye et al. 2016). A promising method of preparing homemade biochar with Kon-Tiki flame curtain pyrolysis, followed by nutrient enrichment has been gaining attention (Schmidt and Taylor 2014). This method follows the principle of pyrolyzing biomass layer after layer in an open, conically built metal kiln (or dug pit) that is easy to operate, fast, and results in high-quality biochar with low greenhouse gas emissions (Cornelissen et al. 2016). Following the pyrolysis, the resulting biochar is steam-activated by quenching with either water or nutrient-rich solutions like urine or cattle slurry, when the biochar is still hot. The activation process increases surface area and porosity, which promote the adsorption of nutrients (Borchard et al. 2012). Schmidt et al. (2015) found that biochar produced with a Kon-Tiki kiln and enriched with cattle urine increased pumpkin yield by 300% compared to urine only treatment, and by 85% compared to same amount of biochar without urine in a silt loam soil. Similarly, Pandit et al. (2017) reported that hot nutrient-enriched biochar produced with a similar flame curtain kilns led to significant increases of 153% in aboveground biomass production of maize compared to cold nutrient-enriched biochar, and 209% compared to biochar added separately from the nutrients during a pot trial.

The application of biochars can affect the N cycling processes (Clough et al. 2013; Nguyen et al. 2017). It has been reported that biochars can reduce mineral N leaching and N₂O emissions, and increase plant N uptake (Borchard et al. 2019;

Tan et al. 2018). However, these beneficial effects are unpredictable because the results presented in the literature are contradictory—some report positive effects, while others report negative or no effect. This suggests that the effects are biochar- and soil-specific (Mia et al. 2017). During field aging, the surface properties of a biochar can change with the development of more oxygen-containing carboxylic functional groups (Cheng et al. 2006), which can increase the retention of NH₄⁺ because of increased cation exchange capacity (Mia et al. 2017). Also, field aging has been reported to enhance the retention of NO₃[−] by physical entrapment into biochar pores (Haider et al. 2016; Joseph et al. 2018). Realizing these benefits of field aging, several techniques of preparing biochar with similar properties as that of field-aged biochar are being explored, such as co-composting (Kammann et al. 2015) and chemical oxidation (Mia et al. 2019). The Kon-Tiki kiln-produced nutrient-enriched biochar may also exhibit promising results because of its higher surface area, porosity, and cation exchange capacity (CEC), resulting from steam activation (Borchard et al. 2012; Cornelissen et al. 2016). However, there is currently no adequate information about the effects of such biochar on the dynamics of applied fertilizer N.

Native soil organic N is also an important source of N for plant productivity. Indeed, the effects of biochar on the dynamics of soil organic matter have been identified as one of the priorities in biochar research (Tammeorg et al. 2017). The small fraction of labile C present in biochar can enhance microbial activity, which can lead to increased mineralization of the soil organic matter (priming effect). Though the reported results vary, some biochars have been shown to induce positive priming, i.e., increased mineralization of soil organic matter (Awad et al. 2012; Wardle et al. 2008), which also increase the mineralization of soil organic N (Nelissen et al. 2012). However, the number of studies on the interactions between biochar application and the dynamics of soil native N is conspicuously low (Fiorentino et al. 2019). Such mineralized N is mostly taken up by microbes (Kuzakov and Xu 2013) and may also get entrapped into biochar pores, which can limit its accessibility for plant uptake. There is limited evidence on whether such additional mineralized N stimulated by biochar addition is available for plant uptake and can thus affect plant productivity in soil-plant system.

Using an innovative ¹⁵N tracing approach with ¹⁵NH₄NO₃ and NH₄¹⁵NO₃ fertilizers, we assessed the effects of two types of biochars—a regular biochar and a Kon-Tiki-produced nutrient-enriched biochar—on the dynamics of fertilizer N. We measured plant fertilizer N uptake and leaching losses of added fertilizer N separately for fertilizer-derived NH₄⁺-N and NO₃[−]-N. We also quantified the importance of fertilizer-derived N versus soil-derived N for the plant yield in the control and biochar-amended soils. We hypothesized that soil amendment with biochars will (1) recover more ¹⁵N in plants and soil with both ¹⁵NH₄⁺-N and ¹⁵NO₃[−]-N fertilization

coupled with reduced NH_4^+ -N and NO_3^- -N leaching and N_2O emissions, as well as (2) promote the uptake of soil-derived N to plant biomass.

Materials and methods

Biochar and soil

The biochars used in this study were a commercially available regular biochar (BC1) and a nutrient-enriched biochar (BC2). Both biochars were produced from wood. The regular biochar (product of RPK Hiili Oy, Mikkeli, Finland) was selected as a representative of commercially available biochars. It was produced by pyrolyzing mixed deciduous wood (hardwood) at 400 °C in a retort kiln. The nutrient-enriched biochar was obtained by pyrolyzing hardwood branches and split logs (approximately 80–90% Willow (*Salix* spp.), 5–10% Birch (*Betula* spp.), and 5–10% other hardwood species: Alder (*Alnus*), Bird cherry (*Prunus padus* L.), and Norway maple (*Acer platanoides* L.)) in a 0.3 m³ Kon-Tiki kiln (<http://terra-magica.info/index.php/terra-magica-kontiki/kon-tiki-garten>). After pyrolysis, the biochar was soaked with a mixture of tap water:cattle slurry (in a ratio of 7:3). Due to technical limitations, the pyrolysis temperature was not measured for the production of BC2. However, it has been reported that the temperature just below flame curtain can reach 680 to 750 °C (Schmidt and Taylor 2014). BC2 has a higher specific surface area and C:N ratio, but lower total N content compared to BC1 (Table 1). The biochar particle size used in the study was 0.2 to 2 mm.

Sandy loam soil (55% sand, 35% silt, and 10% clay, measured with Coulter LS 230 Laser Diffraction Particle Size Analyzer, Beckman Coulter Inc.) was collected from an agricultural field in southwestern Finland (61° 00' 18.5" N, 22° 46' 01.7" E) from a depth of 0–30 cm. The field had been previously planted with barley, peas, and winter wheat for the past 4 years, and fertilized annually with 15 m³ ha⁻¹ pig

slurry. Prior to the experiment, the soil was stored at 5 °C and later sieved through a 4-mm sieve to remove large plant and gravel particles. The soil had pH of 6.9 and electrical conductivity 85 $\mu\text{S cm}^{-1}$ (1:2.5 w/v), and contained 1.06% and 0.10% of total C and N, respectively (measured with Variomax CN analyzer, Elementar Analysensysteme GmbH, Germany).

Experiment

A pot experiment was conducted in a greenhouse at the Viikki Campus, University of Helsinki. The treatments consist of control, fertilizer only, 1% (w/w dry weight basis) BC1 + fertilizer, 1% BC2 + fertilizer, 5% BC1 + fertilizer, and 5% BC2 + fertilizer in a randomized complete block design. ¹⁵N-enriched fertilizers were applied to all five treatments except the control. Each of those five fertilized treatments contained two groups, receiving fertilizers as either ¹⁵NH₄NO₃ or NH₄¹⁵NO₃. Five replicates were included for each group, for a total of 55 pots. The pots (6 × 6 × 6 cm³) with holes at the bottom were filled with moist soil containing approximately 100 g soil on dry weight basis at 50% water holding capacity. The pots were lined with a nylon mesh (50 μm mesh size) at the bottom—this prevented loss of soil through holes while allowing a free flow of water. In the biochar treatments, the soil and corresponding amounts of biochars were mixed properly before filling into the pots. Italian ryegrass (*Lolium multiflorum*) seeds (1 g pot⁻¹, approximately 250 seeds) were spread and gently hand-pressed on top of the soil/soil biochar mixture in every pot. The pots were covered with thin plastic film until germination. For fertilization, 2 mL of 2.5 mg N mL⁻¹ 10 atom% (at%) ¹⁵NH₄NO₃ or NH₄¹⁵NO₃ solution was pipetted over the pots after germination. Each pot was placed inside another, larger pot (9 cm diameter and 6 cm height). This was done to allow watering the plants from below, and for leachate collection during the leaching tests, when water was added on top of the inner pots to simulate rain and allowed to drain through the soil to the bottom of the

Table 1 Physico-chemical properties of regular biochar (BC1) and Kon-Tiki-produced nutrient-enriched biochar (BC2)

Properties	Regular biochar (BC1)	Nutrient-enriched biochar (BC2)	Method
pH	10.0	9.9	1:2.5 biochar:water (w/v)
EC (mS cm ⁻¹)	1.4	1.2	1:2.5 biochar:water (w/v)
Specific surface area (m ² g ⁻¹)	8–24*	199	N ₂ adsorption
Ash content (%)	6.4	9.1	Gravimetric method (ashed at 500 °C for 3 h)
C (%)	83.4	85.9	VarioMax CN analyser
N (%)	1.4	0.3	VarioMax CN analyser
C:N	60	266	

*The specific surface area of BC1 is referenced from Hellstedt et al. (2018)

outer pot. The plants were watered by pouring about 10–40 mL of N-free reverse osmosis water frequently (usually every 2 days) in the bottom of the outer pot. Usually, after 2 days, there was no water remaining in the outer pots, although some pots occasionally had some water. The volumes of water used for watering differed due to the varying amount of water that remained from previous watering. Also, during later phases of the experiment, more water was required to supplement the growing plants. For the measurement of soil pH, soil and biochar were mixed separately in the same ratios as in the planted pots, but fertilizer was not added. The pH of those soil and soil biochar mixtures was measured by a standard combination electrode in a 1:2.5 (w/v) suspension in Milli-Q water. The average daily temperature inside the greenhouse was 18.4 °C and average daily relative humidity was 71%.

Sampling and measurements

Leaching test

The leaching test was carried out on days 4, 12, 17, and 24 after the application of ^{15}N -labelled fertilizers. We aimed at collecting about 40 mL of leachate for later analysis of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations and their ^{15}N content; thus, the amounts of water used for the leaching test were adjusted during the experiment. The leaching test was also carried out 2 days after adding the fertilizer, but since a very small amount of leachate was collected from the 5% biochar treatments, the leachate collected on day 2 was combined with that of day 4 for analysis. Before conducting the leaching test, any remaining water (from the previous watering) on the bottom of the outer pot was discarded, to allow us to quantify the volume of water that leached through the soil. For leaching, about 45–60 mL of reverse osmosis water was poured on top of the soil in the inner pot. The volume of water used was the same for all the pots each time a leaching test was conducted, but was adjusted during the experiment to allow ca. 40 mL of leachate to be collected at all times, as explained above. The exact volume of water added was recorded. The added water was allowed to leach through the soil for ca. 30 min and then collected from the bottom of the outer pot. The volume of this leachate was measured, and the leachate was frozen at $-20\text{ }^\circ\text{C}$ until further analyses. To determine $^{15}\text{NH}_4^+\text{-N}$ and $^{15}\text{NO}_3^-\text{-N}$ in the leachate, the leached $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ were concentrated on acidified filter paper prior to analysis (Sørensen and Jensen 1991). The ^{15}N contents on the filter papers were measured by elemental analysis (CE 1110, Thermo Electron, Milan, Italy) coupled in continuous flow mode to a Finnigan MAT Delta PLUS isotope ratio mass spectrometer (IRMS; Thermo Scientific, Bremen, Germany). The mineral N concentration in the leachate was determined with an automated colorimetric method [“Ammonia (DIC)” for $\text{NH}_4^+\text{-N}$ and

“TON-V” for $\text{NO}_3^-\text{-N}$] using Gallery™ Plus Discrete Analyzer (Thermo Scientific™, Vantaa, Finland).

The initial plan was to measure ^{15}N in the leachate throughout the experiment. However, the concentration of N in leachates was very low, apart from the first leaching test conducted on days 2 and 4 after fertilization. Hence, only the leachate collected on days 2 and 4 after fertilization (combined to allow sufficient volume) was analyzed for ^{15}N ; in the remaining sampling periods, the amounts of N in the leachate were under the detection limit for ^{15}N analysis. Accordingly, we present the ^{15}N results for the first leaching test (day 2 + day 4) only; however, the concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ are presented for all the leaching tests, and all time points were used to calculate the cumulative $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ leaching.

Greenhouse gas measurement

For measuring the GHG emissions, each pot was placed inside a glass jar (3.1 L volume) with an air-tight nozzle fixed in its lid for gas sampling. After over-pressurizing the glass jar with 80 mL of ambient air, 20-mL gas samples were taken into 12 mL helium flushed evacuated Exetainers® (Labco Scientific, High Wycombe, UK) at 0, 4, 20, and 24 h after closing the jars. The concentrations of GHGs (CO_2 , CH_4 , and N_2O) in the gas samples were measured using a gas chromatograph (7890A, Agilent Technologies, California, USA) equipped with a flame ionization detector (FID) and a methanizer for CO_2 and CH_4 , and an electron capture detector (ECD) for N_2O (Pihlatie et al. 2013). The GHG emission rate was calculated by fitting linear regression to the measured gas concentrations over measurement time. The GHG measurements were carried out from only three replicates. The presented GHG results were measured 1 day after fertilizer application. Since the ryegrass seeds were just germinated at that time, the contributions of photosynthesis and plant respiration to the CO_2 flux measurement were considered negligible and hence the measured CO_2 emissions represent only the respiration of soil or soil biochar mixture.

Plant and soil sampling

At the end of the experiment (33 days after sowing), plants and soils were destructively sampled. The aboveground biomass was separated by cutting the ryegrass at the soil surface, and the roots were separated from the growing media by washing with water. The plant samples were dried at $60\text{ }^\circ\text{C}$ for 48 h to calculate aboveground biomass (AGB) and root biomass. The plant samples (aboveground and root) and soil samples were finely ground using a ball-mill and analyzed for ^{15}N content with an elemental analyzer coupled in continuous flow mode to a Finnigan MAT Delta PLUS isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany).

Calculation and data analysis

The ^{15}N content in plant, soil, and leachate derived from added labelled fertilizer was calculated using the so-called N derived from fertilizer (Ndff) equation (Powelson and Barraclough 1993):

$$\text{Ndff}^{15}\text{NH}_4^+ = \frac{T(A_s - A_b)}{A_a} \quad (1)$$

$$\text{Ndff}^{15}\text{NO}_3^- = \frac{T(A_s - A_b)}{A_n} \quad (2)$$

$$\text{Ndff} = \text{Ndff}^{15}\text{NH}_4^+ + \text{Ndff}^{15}\text{NO}_3^- \quad (3)$$

$$\text{Nds} = T - \text{Ndff} \quad (4)$$

where T is the total N content in the samples (plant/soil/leachate); A_s is at% ^{15}N excess of the sample (plant/soil/leachate); A_b is at% ^{15}N excess of the control (without receiving ^{15}N fertilizer); A is at% ^{15}N excess of $^{15}\text{NH}_4\text{NO}_3$ or $\text{NH}_4^{15}\text{NO}_3$; $\text{Ndff}^{15}\text{NH}_4^+$ is N derived from $^{15}\text{NH}_4\text{NO}_3$; $\text{Ndff}^{15}\text{NO}_3^-$ is N derived from $\text{NH}_4^{15}\text{NO}_3$; Ndff is N derived from added fertilizer; and Nds is N derived from soil, which accounts for N originated from soil + seed (+ biochar) mixture $\text{Ndff}^{15}\text{NH}_4^+$ was calculated from the samples with $^{15}\text{NH}_4\text{NO}_3$ fertilization and $\text{Ndff}^{15}\text{NO}_3^-$ was calculated from the samples with $\text{NH}_4^{15}\text{NO}_3$ fertilization.

For the mass balance of the added ^{15}N , ^{15}N recovery and unaccounted ^{15}N were calculated. The recovered ^{15}N was calculated as the sum of the ^{15}N retained in the soil and taken up by plants. Unaccounted ^{15}N was the difference between the total ^{15}N applied and ^{15}N recovered, which corresponds to the lost ^{15}N either through leaching or gaseous losses. The N content derived from overall NH_4NO_3 in a pool was the sum of the average ^{15}N recovered from $^{15}\text{NH}_4\text{NO}_3$ fertilization and average ^{15}N recovered from $\text{NH}_4^{15}\text{NO}_3$ fertilization in that pool for the corresponding treatments (Eq. 3).

The fertilizers applied were both ammonium nitrate ($^{15}\text{NH}_4\text{NO}_3$ and $\text{NH}_4^{15}\text{NO}_3$) and contained the same weight of N, with the only difference of ^{15}N being linked in different moieties. Hence, while analyzing the data when there is no relevance of ^{15}N , both fertilizers were regarded as the same. This resulted in 10 replicates for a given treatment except for the control (which has 5 replicates). The effects of the treatments were tested with one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine the differences among the level of treatments. The residual normal distribution and homogeneity of variances were ascertained by plotting residuals of the model against theoretical quantiles and fitted values, respectively. Whenever relevant, a t -test was carried out to compare the means between two specific categories for better contrast, for example, biochar vs. without biochar treatments, 1% biochar vs. 5% biochar treatments, and BC1 vs. BC2 treatments. The data was

analyzed at 5% level of significance. For Ndff and Nds in plant biomass, the standard deviations were calculated using propagation of uncertainty. The means of Ndff and Nds among the treatments were compared using a pairwise t -test. The statistical analysis was carried out using R version 3.5.3 (R Core Team 2018).

Results

Plant biomass and soil pH

Compared to the fertilized control, the 1% biochar treatments had no effect, while the 5% biochar treatments significantly increased aboveground biomass (Fig. 1a). The 5% BC1 and 5% BC2 treatments increased the aboveground biomass by 22% and 23% respectively compared to the fertilizer only treatment. At the corresponding biochar application rates, there was no difference in aboveground biomass between BC1 and BC2. No differences across treatments were found in terms of root biomass yield (Fig. 1b). The addition of both biochars at 1% application rate significantly increased soil pH from 6.9 to 7.2 ($p < 0.05$). The addition of both biochars at 5% application rate significantly increased soil pH further to 7.5 ($p < 0.05$). Soil pH was similar in BC1 and BC2 at respective application rates.

Leaching

The biochar treatments reduced the volume of leachate collected. The 5% biochar treatments were more efficient than the 1% biochar treatments in reducing the leaching of water through soil. The 5% BC2 significantly reduced leachate volume in day 2 + day 4 after fertilizer N application compared to 5% BC1. Similarly, in day 17 after fertilizer N application, 1% BC2 had significantly lower leachate volume compared to 1% BC1 (Supplementary Fig. 1).

The concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in leachate were significantly lower in biochar treatments relative to the fertilizer only treatment during the first (day 2 + day 4) leaching test (Table 2). However, no differences were observed between biochar treatments and the fertilizer only treatment in the later leaching tests (days 12, 17, and 24 after fertilization). When comparing the biochar treatments at 1% application rate, BC1 had significantly higher $\text{NH}_4^+\text{-N}$ concentration in the leachate during the first leaching test compared to BC2. When looking at the effects of biochar application rates, we found that 5% BC1 had significantly lower $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentration in the leachate compared to 1% BC1 on day 24.

The biochar treatments significantly reduced cumulative $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ leaching compared to fertilizer only treatment (Table 2). The biochar treatments reduced total

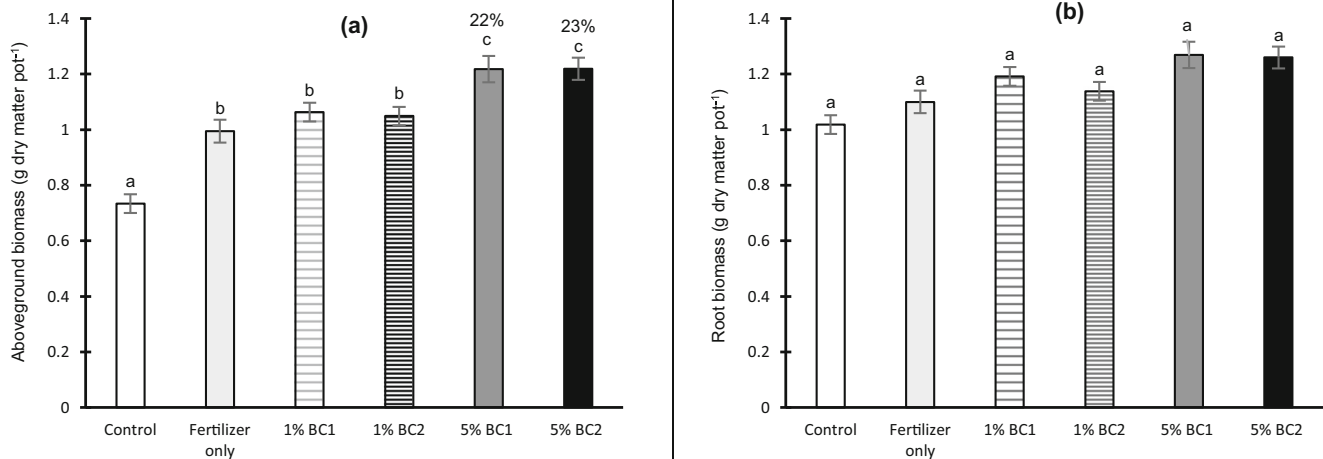


Fig. 1 Ryegrass aboveground (a) and root (b) biomass yield (Mean ± SE) across the treatments. Different letters represent significant differences between treatments ($p < 0.05$) indicated by Tukey's test. The percentages above the bars represent the relative change compared to

NH_4^+ -N leaching by 21–53% and total NO_3^- -N leaching by 47–68%. At 1% biochar application rate, BC1 had significantly higher cumulative NH_4^+ -N leaching compared to BC2, while no differences were observed at 5% biochar application rate. There were no statistical differences in cumulative NO_3^- -N leaching between the two biochar treatments at either of the application rates.

Greenhouse gas emissions

Immediately following fertilizer application, the biochar treatments reduced N_2O emissions compared to the fertilizer only treatment by 57–81% (average 69%). However, no differences in N_2O emissions were observed between the biochar treatments (Fig. 2a). N_2O emissions were negligible from the non-fertilized control pots. On the other hand, 1% BC1 treatment significantly increased CO_2 emission rate by 34% (Fig.

2b) and overall, biochar treatments had higher average CO_2 emissions compared to the treatments without biochar ($t = 3.23$, $p < 0.01$). There were almost no CH_4 emissions from any of the treatments (data not shown).

Distribution of added ^{15}N

When the fertilizer was applied in the form of $^{15}\text{NH}_4\text{NO}_3$, biochar treatments significantly reduced the recovery of ^{15}N in ryegrass biomass compared to the fertilizer only treatment (Fig. 3a). In contrast, when the fertilizer was applied in the form of $\text{NH}_4^{15}\text{NO}_3$, the biochar treatments significantly increased the recovery of ^{15}N in ryegrass biomass compared to the fertilizer only treatment (Fig. 3b). There were no statistically significant differences in the ^{15}N amounts retained in the soil (Fig. 3c and d), even though with $^{15}\text{NH}_4\text{NO}_3$ fertilization, there was a tendency for less ^{15}N to be retained in the soil of

Table 2 Mineral N concentration in leachate and cumulative mineral N leached

	Concentration in leachate ($\mu\text{g L}^{-1}$)								Cumulative N leached ($\mu\text{g N pot}^{-1}$)	
	NH_4^+ -N				NO_3^- -N				NH_4^+ -N	NO_3^- -N
Treatments	Day 2 + day 4	Day 12	Day 27	Day 24	Day 2 + day 4	Day 12	Day 27	Day 24		
Control	43.24 ^{ab}	22.30 ^a	37.99 ^a	34.17 ^{ab}	267.02 ^a	108.51 ^a	144.57 ^a	136.53 ^{ab}	5.97 ^{ab}	28.57 ^a
Fertilizer only	133.97 ^c	36.49 ^b	22.08 ^a	37.61 ^{ab}	1285.04 ^b	184.53 ^b	158.89 ^a	146.87 ^b	8.46 ^b	63.10 ^b
1% BC1	82.19 ^b	43.35 ^b	25.72 ^a	40.73 ^b	498.23 ^a	171.61 ^b	137.99 ^a	142.42 ^b	6.68 ^b	33.21 ^a
1% BC2	29.07 ^a	38.77 ^{ab}	24.10 ^a	24.28 ^{ab}	190.80 ^a	152.73 ^{ab}	136.44 ^a	123.56 ^{ab}	4.21 ^a	27.86 ^a
5% BC1	39.53 ^{ab}	34.19 ^{ab}	21.61 ^a	17.24 ^a	308.20 ^a	175.99 ^b	136.17 ^a	110.44 ^a	3.92 ^a	21.75 ^a
5% BC2	34.15 ^{ab}	33.18 ^{ab}	30.06 ^a	24.94 ^{ab}	187.70 ^a	162.68 ^{ab}	134.32 ^a	119.23 ^{ab}	4.00 ^a	20.15 ^a

Different letters across treatments indicate significant differences between treatments ($p < 0.05$). The biochar treatments (1% BC1, 1% BC2, 5% BC1, and 5% BC2) received the same fertilization as the fertilizer only treatment

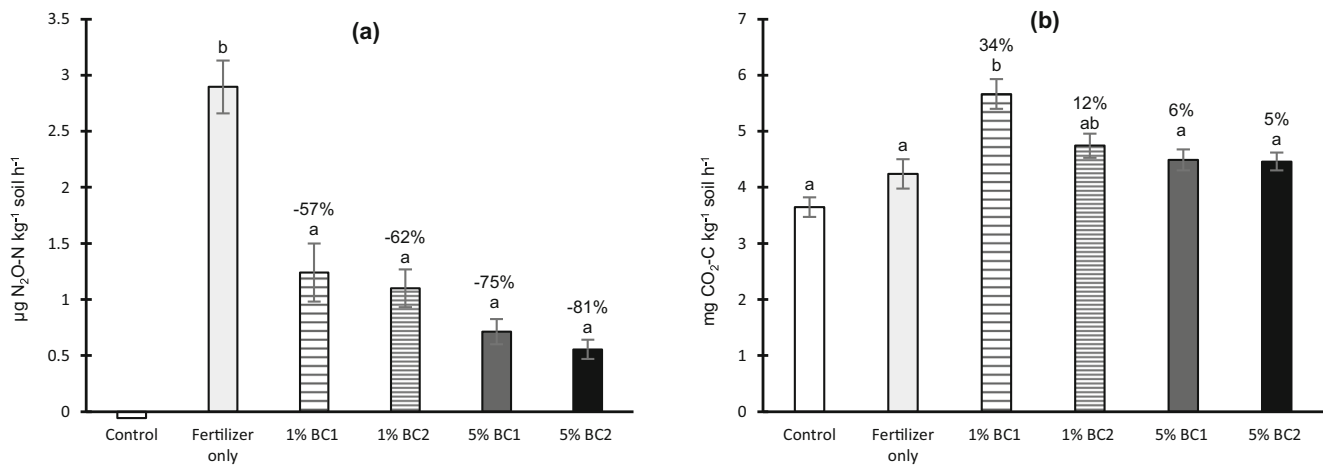


Fig. 2 Emissions of N₂O (a) and CO₂ (b) across the treatments (Mean \pm SE). Different letters represent significant differences across treatments ($p < 0.05$) indicated by Tukey's test. The percentages above the bars represent the relative change compared to fertilizer only treatment. The

the biochar treatments compared to the fertilizer only treatment, especially at the higher 5% biochar application rate (Fig. 3c).

No differences were found in $^{15}\text{NH}_4^+$ -N leaching across all treatments in both fertilization types (Fig. 3e and f). However, there was high variation in $^{15}\text{NH}_4^+$ -N leaching within the fertilizer only treatment. The average $^{15}\text{NH}_4^+$ -N leaching was ca. 9 times higher than that of the average of the biochar treatments when fertilizer was applied as $^{15}\text{NH}_4\text{NO}_3$ (Fig. 3e). The 5% BC1, 5% BC2, and 1% BC2 treatments had significantly lower $^{15}\text{NO}_3^-$ -N leaching compared to the fertilizer only treatment, when fertilizer was applied as $^{15}\text{NH}_4\text{NO}_3$ (Fig. 3g). When fertilizer was applied as $\text{NH}_4^{15}\text{NO}_3$, the observed pattern was the same, but only the 5% BC2 treatment had significantly reduced $^{15}\text{NO}_3^-$ -N leaching compared to the fertilizer only treatment, due to higher variability within treatments (Fig. 3h). When compared to the fertilizer only treatment, $^{15}\text{NO}_3^-$ -N leaching tended to be reduced in BC2 more than the BC1 treatments, but the differences between the two biochar treatments were not statistically significant. It is notable that the amounts of $^{15}\text{NO}_3^-$ -N leaching were almost identical regardless of whether the fertilizer was applied as $^{15}\text{NH}_4\text{NO}_3$ or $\text{NH}_4^{15}\text{NO}_3$. This indicates a quick transformation of $^{15}\text{NH}_4^+$ -N into $^{15}\text{NO}_3^-$ -N, and that the N was mostly leached in $^{15}\text{NO}_3^-$ -N form in both fertilization types.

The total plant N uptake (mg N pot^{-1}) was significantly higher in the 5% biochar treatments compared to the fertilizer only treatment (Fig. 4). The ^{15}N analysis revealed that there was no difference in the total amount of N derived from added NH_4NO_3 fertilizer in plant biomass between the different treatments, because the higher plant uptake of $^{15}\text{NO}_3^-$ -N in the biochar treatments compared to the fertilizer only treatment was offset by the lower $^{15}\text{NH}_4^+$ -N uptake. However, the 5% biochar treatments significantly increased the amount

of N derived from soil (or soil + biochar mixture) by about 24% compared to the fertilizer only treatment (Fig. 4). Both the average amount of soil-derived N in the plant biomass as well as plant biomass yield increased in the order – control < fertilizer only < 1% biochar treatments < 5% biochar treatments. There was a strong positive relationship between the N derived from soil and plant biomass yield ($R^2 = 0.85$, $p < 0.05$, Fig. 5), but there was no relationship between total N derived from fertilizer NH_4NO_3 and plant biomass yield ($R^2 = 0.01$, $p > 0.05$).

The biochar treatments increased the loss of added $^{15}\text{NH}_4^+$ -N, but reduced the loss of added $^{15}\text{NO}_3^-$ -N (Supplementary Table 1). Most of the total applied ^{15}N was recovered in plant biomass and soil (84–86%) while a rather small fraction was lost (14–16%) in all treatments. The amount of ^{15}N lost via leaching in the first leaching test (day 2 + day 4) was less than 0.1% of the total applied ^{15}N in all fertilized treatments.

Discussion

Biochar reduced soil mineral N leaching and N₂O emission

In agreement with earlier lysimeter studies (Lehmann et al. 2003; Xu et al. 2016), we found that biochar treatments reduced the concentrations and total cumulative amounts of both NH_4^+ -N and NO_3^- -N in leachate compared to the fertilizer only treatment (Table 2). In addition, biochar treatments reduced the fertilizer-derived $^{15}\text{NH}_4^+$ -N (though not statistically significant) and $^{15}\text{NO}_3^-$ -N in leachate compared to the fertilizer only treatment (Fig. 3e–h). The reduction in leaching of NH_4^+ -N in biochar treatments could be due to increased sorption of NH_4^+ -N into increased cation exchange sites aided

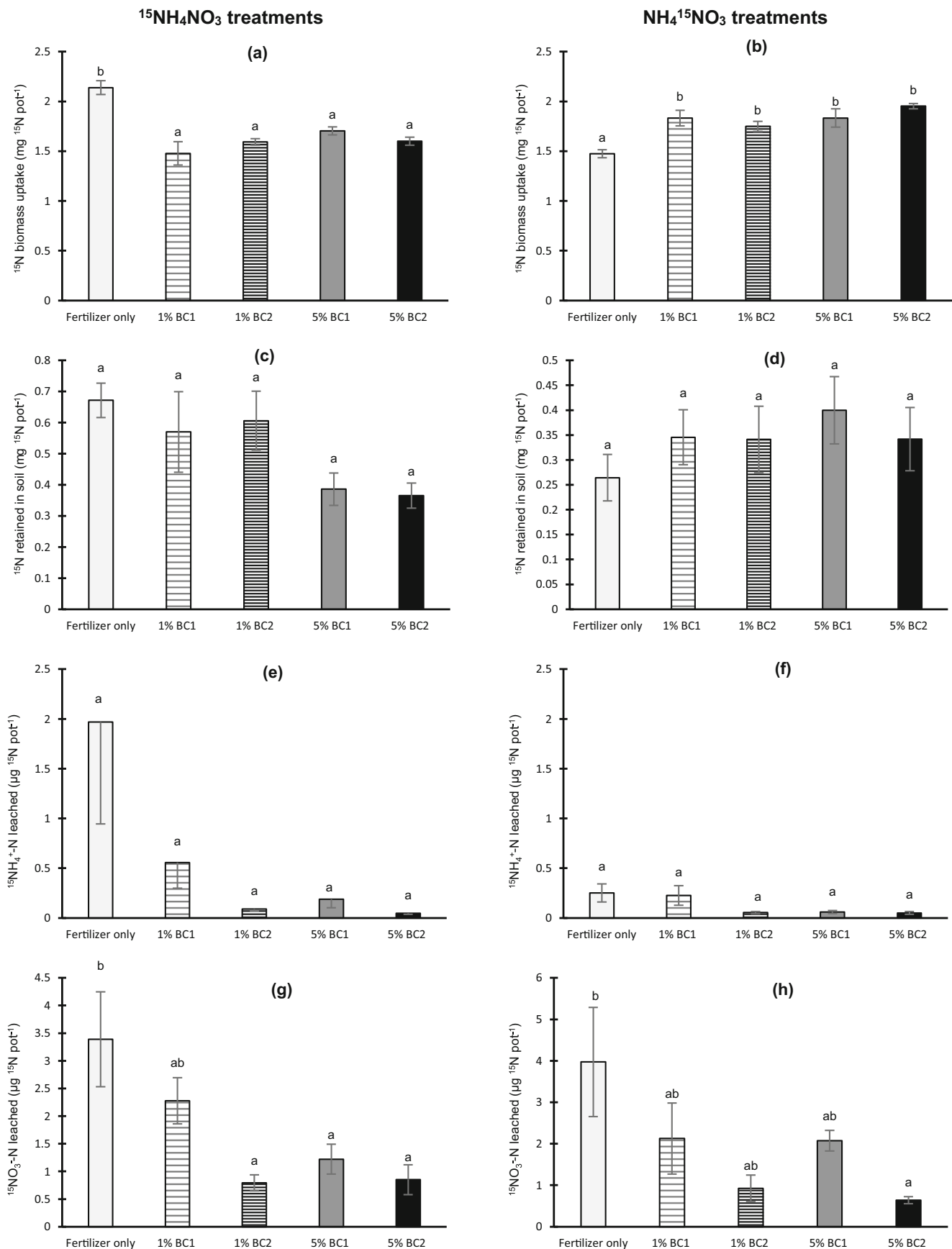


Fig. 3 Distribution of ^{15}N (Mean \pm SE) in biomass (a, b), soil (c, d), and leachate (e–h). The ^{15}N leached data presented is from leachate collected during day 2 + day 4. The left column represents the distribution of ^{15}N from the $^{15}\text{NH}_4\text{NO}_3$ treatments and the right column represents the distribution of ^{15}N from the $\text{NH}_4^{15}\text{NO}_3$ treatments. Different letters across treatments represent significant differences between treatments ($p < 0.05$) indicated by Tukey's test. The biochar treatments (1% BC1, 1% BC2, 5% BC1, and 5% BC2) received the same fertilization as the fertilizer only treatment. For all the treatments, $n = 5$ if there were no missing data

by biochar addition (Gai et al. 2014; Kizito et al. 2015; Liang et al. 2006). Although there were no differences in the leaching of fertilizer $^{15}\text{NH}_4^+\text{-N}$ between the two biochars (Fig. 3e), BC2 was more efficient in reducing the total cumulative $\text{NH}_4^+\text{-N}$ leaching compared to BC1 at 1% application rate (Table 2). This was likely because of the higher adsorption of $\text{NH}_4^+\text{-N}$ associated with the higher specific surface area in BC2 (about 8-fold higher than BC1, Table 1), which might have resulted from the steam activation after the pyrolysis.

Since biochars usually have limited affinity to adsorb $\text{NO}_3^-\text{-N}$ (Gai et al. 2014; Yao et al. 2012), the reduced $\text{NO}_3^-\text{-N}$ leaching may be due to its entrapment inside the biochar pores (Haider et al. 2016; Kammann et al. 2015), because of strong capillary action. Such entrapment is mostly because of physical rather than chemical processes, and is thus affected by the surface area of biochar (Yang et al. 2017), as a higher surface area provides more micropores. Accordingly, during the first leaching test (day 2 + day 4), there was an indication that BC2 reduced $^{15}\text{NO}_3^-\text{-N}$ leaching more than BC1 (Fig. 3g–h), even though this difference was not statistically significant. The reduction in mineral N leaching may have also resulted from the reduction in hydraulic

conductivity and thus enhanced retention of water (Supplementary Fig. 1) in biochar micropores, and changes in pore-size distribution (Glaser et al. 2002). The reduced leaching of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ in biochar treatments was observed only during the first leaching test when the ryegrass plants were still small. In later leaching tests, the differences in $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ concentrations of leachates between fertilizer only and biochar treatments became smaller, most likely because the otherwise leachable, plant-available mineral N had already been taken up by the ryegrass.

The N_2O emission from the control treatment was almost non-existent, and therefore, we can conclude that the observed N_2O emissions from fertilizer only and biochar treatments originated from the added fertilizer N. The biochar treatments reduced N_2O emission by an average of 69% immediately after fertilization—a much greater N_2O emission reduction with biochar than reported in the recent meta-analyses (6–38%) (Borchard et al. 2019; Liu et al. 2019). Our experimental design did not allow detailed studies on the mechanisms underlying the reduction of N_2O emission. However, based on previous studies, it is possible that the reduced N_2O emissions in biochar treatments might be a result of the limited accessibility of $\text{NO}_3^-\text{-N}$ retained in the pores of biochar for denitrification (Cayuela et al. 2013; Haider et al. 2016; Van Zwieten et al. 2014). In addition, through the increased soil pH, biochar could have promoted complete denitrification from $\text{NO}_3^-\text{-N}$ to N_2 instead of N_2O as the end product of denitrification (Cayuela et al. 2013; Dannenmann et al. 2018; Harter et al. 2013; Sánchez-García et al. 2014). The biochar treatments at both application rates had a pH higher than 7, which favors the synthesis and assembly of N_2O reductase, promoting N_2 as the final product of denitrification over N_2O (Bergaust et al. 2010). The lower N_2O emission might also result from

Fig. 4 N derived from fertilizer (light grey) and N derived from soil (dark grey) in plant biomass (Mean \pm SE). Different lowercase letters across the treatments indicate significant differences in N derived from soil or N derived from fertilizer between the treatments. Different uppercase letters above the graph indicate significant differences in total N uptake between the treatments. The percentage represents the relative change compared to fertilizer only treatment. The biochar treatments (1% BC1, 1% BC2, 5% BC1, and 5% BC2) received the same fertilization as the fertilizer only treatment

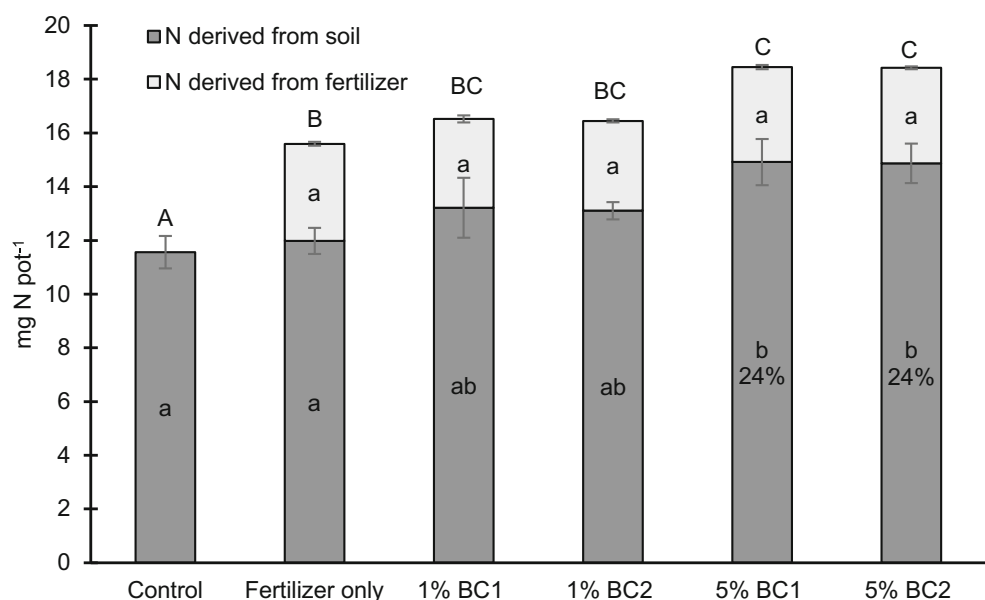
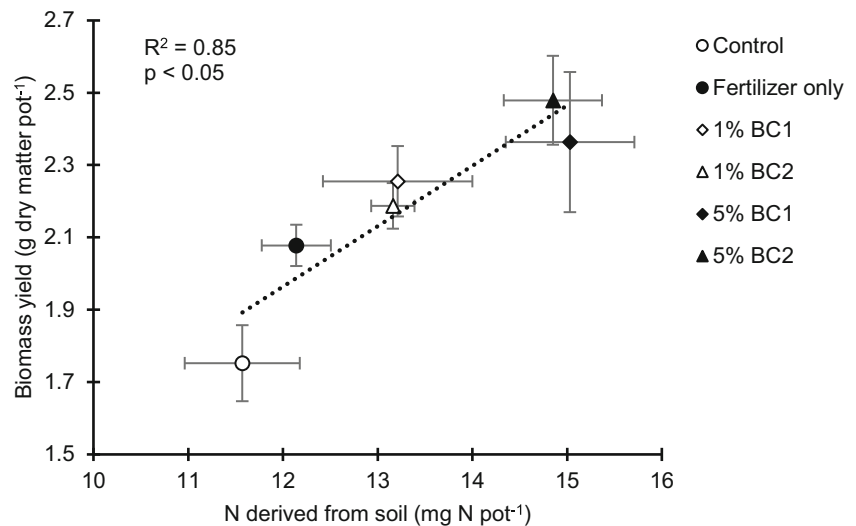


Fig. 5 Relationship between plant biomass yield (aboveground + root biomass) and N derived from soil (Mean \pm SE). The biochar treatments (1% BC1, 1% BC2, 5% BC1, and 5% BC2) received the same fertilization as the fertilizer only treatment



increased immobilization of added N (Baggs et al. 2006; Lan et al. 2017). However, we found no difference in the N_2O emissions between the two types of biochars even though the C:N ratio of BC2 was much higher than that of BC1, with a higher potential for N immobilization. Our finding is similar to the result obtained by Cayuela et al. (2013), who reported no relationship between C:N ratios of biochars and N_2O mitigation, suggesting that microbial N immobilization may not be the main driving mechanism behind reduced N_2O emission.

Biochar enhanced the recovery of added $^{15}\text{NO}_3^-$ -N, but reduced that of added $^{15}\text{NH}_4^+$ -N

The recovery of ^{15}N in plant biomass was decreased by biochar treatments when the added ^{15}N was in the form of $^{15}\text{NH}_4^+$ -N. On the other hand, the ^{15}N recovery increased when it was in $^{15}\text{NO}_3^-$ -N form. This was partly contradictory to our hypothesis 1, since we had expected that biochar would increase the recovery of both mineral N forms in plant biomass. Similarly, the recovery of ^{15}N in soils, when added in $^{15}\text{NH}_4^+$ -N form, tended to be lower in the biochar treatments than in the fertilizer only treatment, especially at the higher 5% biochar application rates, but these differences were not statistically significant. This is contradictory to the assumption that increasing the CEC of soil with the addition of biochar would help to retain more $^{15}\text{NH}_4^+$ -N in plant available form, and thus increase its plant uptake. Instead, these results suggest that biochar stimulated the loss of $^{15}\text{NH}_4^+$ -N, and increased the retention of $^{15}\text{NO}_3^-$ -N in soil. One of the pathways for loss of $^{15}\text{NH}_4^+$ -N could be the conversion of $^{15}\text{NH}_4^+$ -N to $^{15}\text{NO}_3^-$ -N through nitrification followed by $^{15}\text{NO}_3^-$ -N leaching and gaseous N losses: mostly as N_2O during nitrification, and N_2O and N_2 during denitrification (Huber et al. 1977). The recovery of $^{15}\text{NO}_3^-$ -N in the leachate in $^{15}\text{NH}_4\text{NO}_3$ treatments with their magnitudes as high as that

in $\text{NH}_4^{15}\text{NO}_3$ treatments (Fig. 3g and h) indicates that part of the added $^{15}\text{NH}_4^+$ -N was converted to $^{15}\text{NO}_3^-$ -N (through nitrification), which is in line with the findings that biochar can stimulate the nitrification process (Berglund et al. 2004; Dannenmann et al. 2018). Nevertheless, we found that the application of biochars helped to reduce NO_3^- -N leaching and N_2O emission. It is also unlikely that significant amounts of added $^{15}\text{NH}_4^+$ -N were lost during denitrification as N_2 , because if that had been the case, we would have observed the loss of added $^{15}\text{NO}_3^-$ -N in the presence of biochar in the $^{15}\text{NO}_3^-$ -N fertilized treatments too. As such, the loss of added $^{15}\text{NH}_4^+$ -N through biochar-induced nitrification-denitrification pathway seems implausible. Rather, the loss of added $^{15}\text{NH}_4^+$ -N might be due to ammonia (NH_3) volatilization. The increased soil pH with the addition of biochar with high pH (> 9) and/or combination of biochar with NH_4^+ based N fertilizer might have increased NH_3 volatilization (Mandal et al. 2018; Sha et al. 2019) due to conversion of NH_4^+ -N to NH_3 under high soil pH. Application of 5% biochar increased soil pH more than application of 1% biochar, stimulating higher loss of added $^{15}\text{NH}_4^+$ -N and thus less recovery in soil and plant biomass. As we did not measure NH_3 volatilization in this study, we could not confirm it as the reason behind the loss of added $^{15}\text{NH}_4^+$ -N. Some previous studies have also shown that biochar-induced NH_3 volatilization had led to significant N losses from soil (Schomberg et al. 2012; Sun et al. 2019). Contrary to this, others have outlined the potential of biochar to enhance the adsorption of NH_4^+ and NH_3 , and hence decrease NH_3 volatilization (Mandal et al. 2016; Taghizadeh-Toosi et al. 2012; Sun et al. 2020). Therefore, the effect of a biochar on NH_3 volatilization seems to depend on soil and biochar properties. Thus, further studies measuring NH_3 volatilization in different soil conditions combined with various biochars with different properties would be required before tailored solutions for reducing NH_3 volatilization could be recommended.

When fertilizer was applied in $^{15}\text{NO}_3^-$ -N form, the recovery of ^{15}N in plant biomass and soil was higher in the biochar treatments compared to the fertilizer only treatment as hypothesized. Our findings of the coupling between $^{15}\text{NO}_3^-$ -N loss and its recovery in plant biomass indicate that the $^{15}\text{NO}_3^-$ -N retained in the biochar was safe from leaching and denitrification, but was accessible for plant uptake. Though the values seem comparatively small, $^{15}\text{NH}_4^+$ -N leaching from all treatments with $\text{NH}_4^{15}\text{NO}_3$ fertilization (Fig. 3f) suggests a possible re-mineralization of immobilized $^{15}\text{NO}_3^-$ -N by microorganisms. Nevertheless, a very small amount ($< 0.1\%$) of added ^{15}N was lost through leaching in the first leaching test, and the amount of ^{15}N in the leachates was under the detection limit for ^{15}N analysis in the subsequent leaching tests. This result is similar to the findings of a field ^{15}N tracing experiment, where only about 0.05% of added ^{15}N was recovered in the sub-soil layer suggesting that a very small proportion of the added ^{15}N was lost via leaching (Mia et al. 2017). Such a small amount of added ^{15}N leached indicates that increased plant uptake of added $^{15}\text{NO}_3^-$ -N by biochars was more likely achieved due to reduced loss of added $^{15}\text{NO}_3^-$ -N via gaseous emissions than the reduced leaching of NO_3^- -N in our experiment. In addition, even though biochars reduced the average $^{15}\text{NH}_4^+$ -N leaching, the recovery of added $^{15}\text{NH}_4^+$ -N in soil and plant biomass was decreased because the magnitude of added $^{15}\text{NH}_4^+$ -N lost (most probably via NH_3 volatilization) was much higher than the reduced $^{15}\text{NH}_4^+$ -N leaching.

Biochar increased the uptake of N derived from soil

The addition of biochar did not benefit plant growth through uptake of added NH_4NO_3 because increased plant uptake of the added $^{15}\text{NO}_3^-$ -N was offset by the loss of added $^{15}\text{NH}_4^+$ -N. Instead, addition of biochar at the higher application rate increased the uptake of N derived from soil (or soil + biochar mixture) into plant biomass as expected according to our hypothesis 2. There was a strong positive relationship between soil-derived N in plant biomass and plant biomass yield. Both, the N derived from soil and plant biomass yield increased in the following order: control < fertilized control < 1% biochar treatments < 5% biochar treatments (Fig. 5). This suggests that biochar increased plant biomass yields by increasing the availability of soil-derived (or soil + biochar-derived) N, either by increasing mineralization of native soil organic matter (positive priming effect), and thus releasing organic N into mineral form (Fiorentino et al. 2019; Nelissen et al. 2012; Singh and Cowie 2014), or by acting as a source of N itself. The latter is less likely to be an important mechanism, as it is known that biochar is highly recalcitrant to microbial decomposition, and the amount of N mineralized from biochar itself is usually very small (Fiorentino et al. 2019; Jeffery et al. 2017b).

There was no difference in the soil-derived N uptake into plant biomass between BC1 and BC2 treatments, contrary to

our expectation that BC2 would have increased the availability of plant available N. This suggests that the soaking of biochar with cattle slurry did not load the biochar pores with plant-available N. Although BC2 was enriched with cattle slurry, the total N content of BC2 was lower than that of the BC1, likely because of the difference in feedstock and pyrolyzing temperature. BC2 was produced at a higher pyrolysis temperature than the BC1, and biochars produced at higher temperatures have relatively lower N content (Gai et al. 2014; Mandal et al. 2018). In addition, the amount of N in the cattle slurry mixture used may not have been high enough to increase the N content of BC2 because of high dilution—three parts of cattle slurry were mixed with 7 parts of water to maintain optimum consistency for homogeneous activation of biochar.

Conclusion

Our results suggest that the effect of biochars on the fate of fertilizer N depends on the type of fertilizer N applied. Biochars increased the plant uptake of added NO_3^- -N, which was coupled with reduced NO_3^- -N leaching and N_2O emissions. On the other hand, biochars stimulated the loss of added NH_4^+ -N, which lead to decreased plant uptake. The mechanism behind the loss of added NH_4^+ -N was suspected to be NH_3 volatilization, which needs further confirmation. Thus, our results indicate that, at least in the short-term, the incorporation of NO_3^- -based N fertilizer in combination with wood-based biochars increases soil retention and plant uptake of added fertilizer N. Adding NH_4^+ -based N fertilizer together with such biochars with high pH can increase the risk of fertilizer N loss from soil with neutral or higher initial soil pH. Biochars did not improve the total plant N uptake from fertilizer NH_4NO_3 because increased uptake of NO_3^- -N was offset by reduced uptake of added NH_4^+ -N. Instead, biochars increased the plant biomass yield as a result of increased uptake of soil-derived N. This suggests that biochars could have stimulated the mineralization of soil organic N, and kept the mineralized N accessible for plant uptake. Although the two types of hardwood biochars tested in this study were produced using different techniques and constitute varying physico-chemical properties, they did not differ in their effects on plant productivity and dynamics of fertilizer and soil-derived N.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00374-020-01534-0>.

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Author contributions Subin Kalu: conceptualization, data curation, formal analysis, investigation, methodology, writing – original draft preparation, review and editing, visualization. Gboyega Nathaniel Oyekoya: investigation. Per Ambus: investigation, resources, writing – review and editing. Priit Tammeorg: resources, writing – review and editing, validation. Asko Simojoki: resources, writing – review and editing, validation. Mari Pihlatie: resources, writing – review and editing. Kristiina Karhu: conceptualization, funding acquisition, investigation, methodology, resources, supervision, validation, writing – review and editing.

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Compliance with ethical standards

Competing interests The authors declare no competing interests.

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